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Original Article

Lactate peak on brain MRS in children with syndromic mitochondrial diseases

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Abstract

Background: Brain magnetic resonance spectroscopy (MRS) has been reported to be a valuable noninvasive tool in the diagnosis of some rare diseases. In this study, our aim was to assess lactate peak on single-voxel proton MRS in children with syndromic mitochondrial diseases (MDs).
Methods: From March 2004 to November 2010, 14 patients who were diagnosed with syndromic MDs underwent single-voxel proton MRS examination. The volume of interest was positioned on axial magnetic resonance imaging (MRI), and voxels were sampled using short (35 milliseconds), intermediate (144 milliseconds), or long (288 milliseconds) echo times for determination of lactate at 1.33 parts/million.
Results: Twelve of fourteen patients (85.7%) exhibited lactate peaks on the initial single-voxel proton MRS, and all of them showed abnormal MRI findings. The correlations of lactate level in blood and lactate peak on single-voxel proton MRS were inconsistent. Among the 12 patients, eight (66.7%) had corresponding elevated levels of blood lactate, and four (33.3%) had normal levels of blood lactate. Compared with a positive rate of 85.7% for patients with lactate peaks on the single-voxel proton MRS, the positive rates for diagnosing syndromic MDs by using electron microscopic examination of muscle biopsy, oral glucose lactate stimulation test, and blood lactate level were 100%, 91.7%, and 71.4%, respectively.
Conclusion: Lactate acquisition on single-voxel proton MRS provides a noninvasive and complementary tool for the diagnosis of syndromic MDs, especially in children with abnormal signal changes on the brain MRI or a normal blood lactate level.

Keywords: Blood lactate; Children; Lactate peak; Magnetic resonance spectroscopy; Mitochondrial diseases

1. Introduction

Mitochondrial diseases (MDs) are a group of complex and heterogeneous disorders characterized by impaired energy production, which is caused by mutations in mitochondrial and nuclear genes that lead to oxidative phosphorylation dysfunction. Clinical manifestations of MDs in childhood are often nonspecific because symptoms may arise from any organ or system. Although recent advances in genetics have led to the development of diagnostic tools for many MDs, most of the patients present with rather challenging diagnostic dilemmas. Therefore, diagnostic confirmation of a MD is based on a combination of modalities including clinical features, metabolic survey, histopathologic analysis of muscle specimens, neuroradiologic findings, and molecular genetic studies.[1]

In the last decade, the diagnosis of MDs has been greatly enhanced by advances in neuroimaging. Proton (hydrogen-1

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[^{1}H]) magnetic resonance spectroscopy (MRS), which allows *in vivo* investigation of brain metabolism, has proven to be useful in the study of certain rare hereditary brain metabolic disorders such as childhood white matter diseases,[2−5] and brain creatine deficiency syndromes,[6] as well as in the clinical evaluation of phenotypic MDs. The latter includes specific mitochondrial syndromes, i.e. Leigh syndrome,[7] mitochondrial encephalopathy, lactic acidosis, and strokelike episodes (MELAS),[8] chronic progressive external ophthalmoplegia (CPEO),[9] nonsyndromic MDs,[10,11] and the clinical suspicion of MDs.[12] Furthermore, because of the safety and noninvasive nature of MRS, it can be repeatedly applied to monitor the progression and fluctuating course of MDs.[13]

The aim of this study was to assess lactate peak on brain MRS in children with syndromic MDs. This finding was compared with the results of other diagnostic methods relevant to syndromic MDs including blood lactate level, oral glucose challenge test, and histopathologic results.

2. Methods

From March 2004 to November 2010, 14 patients, 7 males and 7 females who were diagnosed with syndromic MDs, underwent MRS examination. The median age at clinical presentation was 12 months, with a range of 1 month to 13 years.

Detailed neurological examination and basic laboratory tests were performed on all patients. The laboratory tests consisted of analysis of arterial blood gas, blood lactate level (normal 3−12 mg/dL), and metabolic surveys including assays of blood amino acids and urinary organic acids. An oral glucose lactate stimulation test (OGLST)[14] and a spinal tap were administrated to patients after obtaining their parents' consent, and the lactate value of cerebrospinal fluid (CSF) (normal 10−25 mg/dL) and assay of CSF amino acids were determined.

Mitochondrial DNA (mt DNA) common point mutations (mt 3243, 8344, 8993, and 9176) and mt DNA deletions were screened. Total DNA was extracted from peripheral blood using the Purgene blood DNA isolation kit (Qiagen, Valencia, CA, USA). The mt DNA point mutation was screened by polymerization chain reaction-restriction fragment length polymorphism method, and mt DNA common deletion was screened by duplex polymerization chain reaction. In cases with the suspicion of Leigh syndrome, mt genes including mt ND2 through mt ND6, NDUFS1, NDUFS3, NDUFS4, NDUFS7, NDUFS8, and the nuclear gene SURF1 were analyzed by direct sequencing. Written informed consents were obtained from patients' parents.

A skeletal muscle biopsy for light microscopic and electron microscopic (EM) examinations was performed. Morphological examination of the skeletal muscle tissue included histochemical stains such as modified Gomori trichrome staining for ragged-red fibers, adenosine triphosphatase staining for assessment of myofibrillar integrity, muscle-type fiber predominance and distribution, and cytochrome *c* oxidase and succinate dehydrogenase staining for oxidative enzymes.

Definite diagnosis of syndromic MDs was made based on the modified MDs criteria by Bernier et al.[15] The major criteria included (1) clinically complete respiratory chain (RC) encephalomyopathy including Leigh syndrome, Alpers disease, lethal infantile MD, Pearson's syndrome, Kearns-Sayre syndrome (KSS), mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS), myoclonic epilepsy with ragged-red fibers (MERRF), neuropathy, ataxia, and retinitis pigmentosa (NARP), mitochondrial neuro-gastro-intestinal encephalomyopathy (MNGIE), Leber hereditary optic neuropathy (LHON), and deafness dystonia syndrome (DDs), or a mitochondrial cytopathy including unexplained combination of multisystemic symptoms, a progressive clinical course with episodes of exacerbation, and other possible metabolic or nonmetabolic disorders excluded by appropriate testing, and (2) molecular identification of a mt DNA mutation of undisputed pathogenicity. The minor criteria included (1) histologically abnormal mitochondrial configurations and/or abnormal subsarcolemmal mitochondrial accumulation of muscle cells, and (2) an abnormal metabolic indicator of OGLST. All patients who fulfilled a definitive diagnosis, i.e. two major criteria or one major criterion plus two minor criteria, were included in this study.

Brain magnetic resonance imaging (MRI) examinations were performed on a 1.5-T (Siemens, Sonata, Germany) or 3.0-T (Philips, Achieva X-series, Nederland). Standard 1.5-T MRI with T1-weighted images [echo time (TE)/repetition time (TR) 11 ms/550 ms], T2-weighted images (TE/TR 93 ms/4000 ms), and fluid-attenuated inversion recovery (FLAIR) (TE/TR/inversion time 110 ms/10000 ms/2250 ms), as well as 3.0-T MRI with T1-weighted images (TE/TR 5 ms/338 ms), T2-weighted images (TE/TR 80 ms/3000 ms), and FLAIR [TE/TR/inversion time (TI) 125 ms/11000 ms/2800 ms] were performed. A volume of interest for MRS was positioned on the axial MRI with one or multiple regions within the basal ganglia or lesion sites. Voxels were sampled using one or multiple echo times, including 35 milliseconds (short), 144 milliseconds (intermediate) or 288 milliseconds (long), with repetition times of 2000 milliseconds and volumes ranging from 1.5 mL to 8 mL. Choice of echo time and region was dependent on the imaging features at the time of the examination. All spectra were reviewed with a special interest in lactate level elevation. Spectra were evaluated by comparing amplitudes of the resonance signals. Peak amplitude was determined by assuming a Lorentzian line shape and evaluating the baseline noise standard deviation. Amplitudes of myo-inositol at 3.56 parts/million (ppm), choline at 3.25 ppm, N-acetylaspartate at 2.01 ppm, and lactate at 1.33 ppm were expressed. A peak at a TE of 35 milliseconds, an inverted doublet at a TE of 144 milliseconds, or a peak at a TE of 288 milliseconds was used to diagnose the acquisition of lactate.

We analyzed the MRS findings in children with syndromic MDs, which were then compared with the results of other diagnostic methods including blood lactate level, OGLST, and histopathologic results.

3. Results

Among 14 patients with syndromic MDs, seven manifested Leigh syndrome, four exhibited MELAS, one presented with

CPEO, one manifested Pearson syndrome, and one exhibited DDs.

As shown in Table 1, 1.5-T and 3.0-T brain MRIs were performed on 7 patients and 7 patients, respectively. Lactate peaks on the initial MRS were detected in 12 patients (12 of 14; 85.7%), of which nine (9 of 12; 75.0%) were in acute deterioration phase of the disease course (Figs. 1 and 2), and three (3 of 12; 25.0%) were in a stationary period of the disease process. All 12 patients had abnormal MRI findings. Two patients without lactate peaks on the initial MRS had variable MRI features, in which there were signal changes over the bilateral lentiform nucleus in one case with DDs and normal MRI feature in one case with CPEO.

Follow-up brain MRS was performed on 4 patients with either disease progression or stationary clinical course. The median duration of follow-up was 4 months, with a range of 3 months to 1 year and 11 months. The lactate peaks in 3 cases became negative: Case 2 with Leigh syndrome was followed up during the course of disease deterioration, Case 4 with Leigh syndrome and Case 13 with Pearson syndrome were examined during the stationary phase of disease course. Case 8 with MELAS had positive lactate peaks on both initial and follow-up MRS.

Comparisons of lactate peaks on MRS and other diagnostic methods for syndromic MDs, including measurement of levels

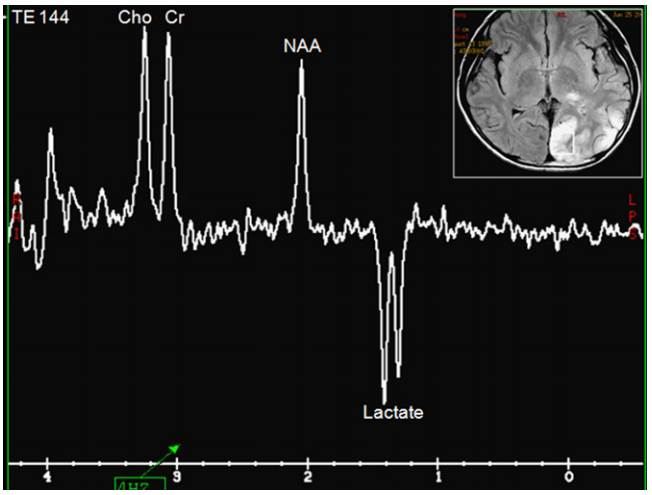
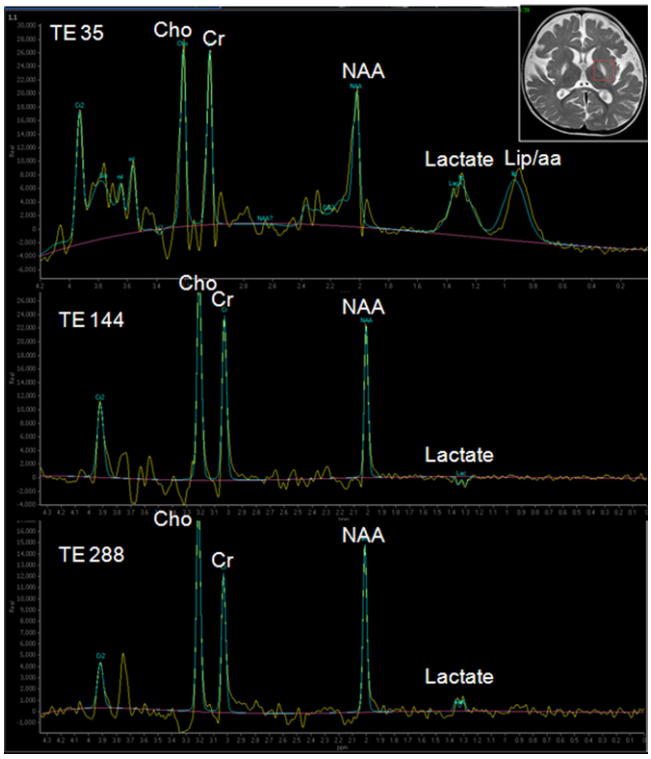


Fig. 1. Lactate peak on brain MRS in mitochondrial encephalopathy lactic acidosis and stroke-like episodes (mt A3243G), Case 10. This 9-year-old boy presents with severe headache, vomiting, right-hand clonic seizures, and short stature. Axial 1.5 T fluid-attenuated inversion recovery imaging (TE/TR/inversion time 120 ms/9002 ms/2250 ms) reveals hyperintensity over the left temporal and occipital areas. A volume of interest is located at the left occipital area. Magnetic resonance spectroscopy using intermediate echo time (144 milliseconds) reveals reduced signals of NAA and presence of an obvious inverted doublet of lactate at 1.33 ppm. Cho = choline, Cr = creatine, NAA = N-acetylaspartate.

Table 1
Summary of clinical diagnosis, brain MRI and brain MRS findings

Pt No.	Age at dz onset	Sex	Clinical diagnosis	MRI findings	MRS (single voxel)		
				Signal changes on T2WI	Lactate peak		Site interrogated
					1st	FU	
1	6 mo	F	LS	Basal ganglia, dorsal aspects of pons and medulla, and cerebellar atrophy	+* (1.5 T) (I)	NA	Right putamen
2	3 mo	M	LS	Bilateral putamen and thalamus	+ (1.5 T) (I)	−* (1.5 T) (I)	Right putamen
3	3 mo	M	LS	Bilateral putamen , dorsal aspects of pons and medulla, and diffuse cerebral and cerebellar atrophy	+* (1.5 T) (I)	NA	Dorsal aspect of pons
4	6 mo	M	LS	Bilateral putamen	+* (3.0 T) (S, I, L)	− (3.0 T) (L)	Left putamen
5	14 mo	M	LS	Bilateral putamen and right globus pallidus	+* (3.0 T) (S, L)	NA	Right putamen
6	4 mo	F	LS	Bilateral putamen, thalamus and brainstem	+* (1.5 T) (I)	NA	Bilateral putamen
7	1 yr 6 mo	F	LS	Bilateral putamen, and caudate nucleus	+* (3.0 T) (S, I, L)	NA	Left putamen
8	7 yr	F	MELAS	Cortex and subcortical white matters of right temporal and parietal areas	+ (3.0 T) (S, I, L)	+* (3.0 T) (S, I, L)	Right parietal lobe
9	9 yr	M	MELAS	Cortex of bilateral temporal and occipital lobes, bilateral subcortical white matter of occipital lobe, and generalized brain atrophy	+ (3.0 T) (S, L)	NA	Right temporal lobe
10	9 yr	M	MELAS	Cortex and subcortical white matter of left parieto-occipital lobe and left thalamus	+* (1.5 T) (I)	NA	Left occipital area
11	5 yr 11 mo	F	MELAS	Cortex and subcortical white matter of bilateral occipital lobes and cortex of bilateral central and parietal areas	+* (3.0 T) (S, I, L)	NA	Left occipital area
12	13 yr	F	CPEO	Normal	− (3.0 T) (S, I, L)	NA	Right putamen
13	1 mo	F	PS	Dorsal aspect of medulla, frontal and parietal gray matter, and cerebellum	+* (1.5 T) (S)	− (1.5 T) (I)	Right frontal gray matter
14	4 mo	M	DDs	Bilateral lentiform nucleus	− (1.5 T) (I)	NA	Right basal ganglia

CPEO = chronic progressive external ophthalmoplegia; DDs = deafness dystonia syndrome; dz = disease; F = female; FU = follow-up; I = intermediate echo time; L = long echo time; LS = Leigh syndrome; M = male; MELAS = mitochondrial encephalopathy lactic acidosis and stroke-like episodes; MRI = magnetic resonance imaging; MRS = magnetic resonance spectroscopy; NA = not available; No. = number; PS = Pearson syndrome; Pt = patient; S = short echo time; T = tesla; T2WI = T2-weighted images; * = MRS shows lactate peak during deterioration of the disease course; "+" = positive finding; "−" = negative finding.



of lactate in blood and CSF, OGLST, histopathological findings, and mt gene analysis, are listed in Table 2. The correlations of lactate levels of blood and CSF and lactate peaks on MRS were inconsistent. Among 12 patients with lactate peaks on MRS, eight (66.7%) had corresponding elevated levels of blood lactate and four (33.3%) had normal levels of blood lactate. Two patients without lactate peaks on MRS showed elevated levels of blood lactate. Spinal tap was conducted on 5 patients with lactate peaks on MRS; two (40.0%) had normal lactate levels of CSF and three (60.0%) had elevated lactate levels.

Our observation showed the positive rates for diagnosing syndromic MDs by using EM examination of muscle biopsy, OGLST, lactate peak on MRS, and blood lactate level were 100%, 91.7%, 85.7%, and 71.4%, respectively.

4. Discussion

The frequency of detectable elevation of lactate on MRS in cases with syndromic MDs or definite MDs reported in the literature ranges from 62.5% (5 of 8)[12] to 81.3% (13 of 16).[11] The detection of lactate peak on MRS is dependent on the timing or severity of the disease, the location of the affected tissues and the site of interrogation, lactate level of CSF, echo times of MRS, or differences in types of MDs.[12,16] In our case series, the acquisition of lactate peaks on MRS was 85.7%. We observed the detection rate of lactate peak was higher at acute deterioration phase of the disease process than at stationary phase of disease course. Our study revealed 66.7% and 60.0% of patients with lactate peaks on MRS had elevated lactate levels of blood and CSF, respectively. On the contrary, cases with normal lactate levels in the blood and CSF still could exhibit lactate peaks on MRS.

It has been reported that lactate peaks on MRS were not found in a normal brain MRI.[17] The result of our study was consistent with that finding, i.e. all 12 cases presented lactate peaks on MRS accompanied with variable specific abnormal MRI features.

Spectra at 3.0 T have been demonstrated to improve sensitivity compared with 1.5 T at short echo time, however, spectra at long echo time exhibited similar signal-to-noise ratio using both 1.5-T and 3.0-T magnets.[18] Measurements of lactate at intermediate echo time on 3.0-T MRS have been reported to show reduced or absent signal intensity, a problem not encountered at 1.5 T.[19] In our study, we observed that Cases 4, 7, 8, and 11 exhibited lactate peaks at short, intermediate, and long echo times, respectively, on 3.0-T magnets. However, Cases 5 and 9 showed lactate peaks at short and long echo times, but none at intermediate echo time.

There is no single algorithm for diagnosing MDs. Several diagnostic methods have been applied to help pediatricians detect a mitochondriopathy earlier. In addition to clinically specific phenotypes, our study showed positive rates of abnormal mitochondrial configurations of muscle biopsy by EM examination and OGLST for detecting syndromic MDs were higher than that of MRS. However, MRS is a noninvasive tool usable in children.

In conclusion, clinical diagnosis in children with mitochondriopathies is not easy because of variable clinical symptoms.[20] MRS examination is a noninvasive complementary tool for helping to corroborate the diagnosis of syndromic MDs, especially in patients exhibiting signal changes on the brain MRI and lactate peaks on MRS but a normal blood lactate level. Furthermore, it helps determining whether further invasive examinations and/or high-cost diagnostic tests such as muscle biopsy and/or genetic analysis, are needed.

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